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Original Article

Sociodemographic characteristics and dynamic thiol/disulfide homeostasis in pediatric patients presenting with scorpion sting

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Abstract

Aim: Poisoning from scorpion sting is an important public health problem, and may cause disability and death especially in childhood. In our study, we aimed to evaluate the sociodemographic characteristics, clinical findings, laboratory results and dynamic disulfide/thiol homeostasis as oxidative stress factor of pediatric patients presenting with scorpion sting complaints to our hospital.

Material and Methods: Forty patients in the 0-18 age group who admitted to the Pediatric Emergency Outpatient Clinic due to scorpion sting intoxication were included in the patient group, and 38 healthy children in the same age group were included in the control group. Patients' sociodemographic characteristics, clinical and laboratory findings, treatment modalities and the outcomes were examined. In addition, thiol/disulfide levels in the serums of patients were studied with a new method developed by Erel et al.

Results: Forty pediatric patients (57.5% male and 42.5% female) presenting with scorpion sting complaints were included in the study. Results: There was no statistically significant difference among patient and control group with regards to the mean native thiol levels. While it was found that the levels of the total thiol, disulfide, disulfide/native thiol and disulfide/total thiol were statistically significant higher in the patient group, the native thiol/total thiol ratio was found to be lower at a significant level.

Conclusion: The scorpion sting incidents are still an important health problem in Turkey. In children with scorpion sting, the thiol/disulfide homeostasis was observed to shift towards the disulfide side, i.e. to the right, indicating an increase in oxidative stress.

Keywords: Pediatric emergency; scorpion sting; dynamic thiol/disulfide homeostasis; oxidative stress.

INTRODUCTION

Scorpions are interesting organisms because of their evolutionary processes, medical importance and the presence of biologically active components in their venom glands (1, 2). Among 1500 different scorpion species known to exist in the world, only 30 species' venom samples have been studied in detail to the present day. Approximately, 100.000 different peptides are estimated to exist in all species, only 0.02% is known (2, 3). Scorpion toxins are mostly effective on excited cells, such as nerves and muscles. Toxins in scorpion venoms mediate effects that can result in death by affecting various neurotransmitters through voltage-dependent Na+ channels and voltage-dependent and other K+ channels (4, 5).

The thiol and disulfide levels, which can be easily measured in the blood by a new method developed by Erel et al. (6), were investigated in various proliferative and inflammatory diseases, and the variables in this balance were observed to correlate with oxidant parameters. Dynamic thiol/disulfide homeostasis play a critical role in the regulation of antioxidant protection, detoxification, signal transduction, apoptosis, enzyme activity and transcription factors and cellular signaling mechanisms (7, 8). In addition, there are ongoing studies carried out to diagnose numerous diseases by evaluating the dynamic thiol/disulfide homeostasis. Determination of dynamic thiol/disulfide homeostasis can provide valuable information about various normal or abnormal biochemical processes.

This study aims to evaluate sociodemographic data of patients admitted due to scorpion sting and the changes dynamic thiol/disulfide homeostasis in this patients.

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MATERIAL and METHODS

Forty patients in the 0-18 age group who admitted to the Pediatric Emergency Outpatient Clinics in the city due to scorpion sting intoxication were included in the patient group, and 40 healthy, voluntary children in the same age group with matching descriptive characteristic were included in the control group in this study. The detailed history of all the children included in the study was taken, and detailed physical and laboratory examinations were carried out. The study was approved by the local ethics committee. Voluntary consent forms were collected after the parents of the children taken to the study were informed about the study.

Sociodemographic, clinical and laboratory findings (complete blood count, C-Reactive Protein (CRP), glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, calcium, creatinine kinase (CK), creatinine kinase–myocardial band (CK-MB), troponin, aspartate amino transferase (AST), alanine amino transferase (ALT)), treatment methods and outcomes were examined. Along with the routine blood samples taken for routine biochemical tests from each patient, 2 cc of blood was taken from the hospitalized patients for determination of levels of dynamic disulfide/thiol homeostasis. The blood samples were centrifuged and stored at -80 °C. At the time of study, thiol/disulfide levels in the blood were easily measured with a new method developed by Erel et al (6).

Exclusion criteria

Children presenting to our hospital with mild local symptoms and acute infections, tuberculosis, congenital anomalies, epilepsy, mental motor retardation, diabetes mellitus, congestive heart failure, chronic renal failure, chronic liver disease, chronic pulmonary disease, hypothyroidism and celiac disease were not included in the study.

Blood samples

Before starting the study, complete blood counts of all patients and children in the healthy control group were taken with the automatic blood count device (Abbott Celldyn 3500 IL, USA). Blood samples taken from the cases selected for research into a dry biochemistry tube were centrifuged for 10 minutes at 3500 rpm, and the formed components were discarded along with the tube. Some of the above serum samples were transferred to the Eppendorf tube and stored at -80 °C. Biochemical parameters from the remaining serum samples were used for biochemical parameters, electrolytes, kidney and liver function tests and CRP (Architect c-16000, Abbott Diagnostics, Abbott Park, IL, USA), hormone tests TSH, free T4 (Siemens, Centaur XP, Ireland) anti-tissue transglutaminase IGA and anti-endomysium IgA (Cobas 8000, Roche) on the same day, while serum stored at -80°C was used on the day of study for the determination of dynamic disulfide/thiol levels. In addition, blood samples were taken to Na-citrate tubes for coagulation tests and studied on the same day (Siemens CS2100i).

Measurement of thiol/disulfide homeostasis parameters

Thiol/disulfide homeostasis tests were measured by a new, fully automated method developed by Erel and Neşelioğlu(6). First, disulfide bonds were reduced to form free functional thiol groups along with sodium borohydride. Unused reducing sodium borohydride was eliminated with formaldehyde in order to prevent the reduction of DTNB (5,5′-dithiobis- (2-nitrobenzoic) acid), all thiol groups including the reduced and native thiol groups were identified after reaction with DTNB. Half of the difference between total thiols and native thiols was calculated as the amount of dynamic disulfide bonding. After the determination of the native and total thiols, the rates of disulfide/total thiol, disulfide/native thiol, native thiol/total thiol and disulfide levels were calculated.

Statistical Analysis

The data obtained in the study were evaluated using the SPSS Version 24 software. The quality assessment, input and analysis of the data were performed by the researcher. The student t test and one-way ANOVA test were used when parametric conditions are met and the Mann-Whitney U test and Kruskall-Wallis test was used for non-parametric conditions in the comparison of various variables with the presenting and pre-discharge clinical and laboratory values. Tukey Test and Bonferoni correction were used in advanced statistical analyses. Linear Regression Analysis was applied in multiple analyses. A statistical significance level of $p<0.05$ was used in the analyses.

RESULTS

A total of 40 patients (23 males (57.5%), 17 females (42.5%)) presenting with scorpion sting complaints was included in the study. The mean age in the patient group was 10.35±4.84 years. Of the 40 patients included in the study as a healthy control group, 26 were males (65%) and 14 were females (35%). The mean age in the control group was 9.92±3.53 years. There was no statistically significant difference between the two groups in terms of age, gender, and BMI ($p>0.05$) (Table 1).

Table 1. Evaluation of demographic characteristics by groups

		Patient (n=40)	Control (n=40)	p
Age (years)	Mean±SD	10.35±4.84	9.92±3.53	0.654
Gender; n (%)	Male	23 (57.5)	26 (65)	0.546
	Female	17 (42.5)	14 (35)	
BMI (kg/m^2)	Mean±SD	17.86±2.26	17.20±2.12	0.166

*p<0.05

Table 2. shows the distribution of the site of stings, presence of local findings, initial admission time and hospitalization time of the pediatric patients in the study. In terms of scorpion sting sites, 11 (27.5%) of the patients were stung from the right foot, and of the remaining pediatric patients, 22.5% was stung from right hand, 17.5% from left foot, and 12.5% from the hip. In terms of the time of admission to the hospital, the majority of patients

Table 2. Sociodemographic and epidemiological characteristics of patients with scorpion sting

		N	%
Gender	Male	23	57.5
	Female	17	42.5
Age (Years)	5 years and under	10	25
	6-10	11	27.5
	11-15	12	30
	16 -18	7	17.5
Patient's education	Illiterate	10	25
	Primary education	16	40
	High School	12	30
	High school graduate	2	5
Residential place	Village	28	70
	City	12	30
Place of sting	Right foot	11	27.5
	Left foot	7	17.5
	Right hand	9	22.5
	Left hand	4	10
	Hip	5	12.5
	Chest	3	7.5
	Abdomen	1	2.5
Hospital arrival time (Hours)	0-3 hours	37	92.5
	3-6 hours	3	7.5
Hospitalization time (Days)	1-2 days	10	25
	3-4 days	21	52.5
	5 days and above	9	22.5

Table 3. Distribution of Presenting Complaints of Pediatric Patients

		N	%
Presenting local findings	Yes	23	57.5
	No	17	42.5
Pain	Yes	32	80
	No	8	20
Vomiting	Yes	4	10
	No	36	90
Edema	Yes	20	50
	No	20	50
Cold extremities	Yes	18	45
	No	22	55
Sweating	Yes	9	22.5
	No	31	77.5
Convulsion	Yes	1	2.5
	No	39	97.5
Priapism	Yes	3	7.5
	No	37	92.5
Blood pressure	Normotensive	28	70
	Hypotensive	10	25
	Hypertensive	2	5
Cardiac examination	Yes	16	40
	No	24	60
State of consciousness	Open	28	70
	Confused	11	27.5
	Coma	1	2.5

(92.5%) were found to admit to the hospital within the first 3 hours after scorpion sting. Of the patients, 25% was found to be hospitalized for 1-2 days, 52.5% for 3-4 days, and 22.5% for 5 days and over. Of the pediatric patients,42.5% had no local findings at the time of admission.

Table 4. Distribution of Treatments Received by the Pediatric Patients during the Clinical Stage and at the Time of Admission

		N	%
Stage	1. Stage	29	72.5
	2. Stage	11	27.5
Dose of antivenom	None	10	25.0
	Single dose	27	67.5
	Multi-dose	3	7.5
Tetanus	None	19	47.5
	Done	21	52.5

Table 5. Laboratory Results of Children with Scorpion Sting

Laboratory Results	mean±SD (min-max)
White blood cell count (/uL)	12.24±4.26 (4.98-24.80)
PNL (/uL)	8.365±4.013(2.43-20.80)
LYM(/uL)	3.0±2.0(0.5-10.8)
Hemoglobin	13.0±1.3(10.2-15.4)
Hemotocrit (%)	38.5±3.3(31.6-45.3)
MCV	76.8±5.8(63.0-87.0)
Platelet count (/uL)	339±89(166-596)
MPV	7.22±1.76 (4.02-14.19)
CRP	0.15±0.25(0.01-0.84)
Glucose	121.10±48.24(67-348)
Urea	23.61±8.43(0.35-55.00)
Creatine	0.6±0.1(0.4-1.3)
Total protein	7.1±0.6(4.9-8.2)
Albumin	4.1±0.3(3.4-4.9)
Total Bil.	0.4±0.2(0.1-1.3)
Direct Bil.	0.2±0.1(0.1-0.4)
ALT	19.07±8.91(6.00-50.00)
AST	34.27±19.25(13.00-135.00)
LDH	314.15±70.06(201.00-459.00)
CK	334.40±493.43(47.00-2646.00)
Ca	12.05±14.92(8.60-104.00)
Na	137.63±2.72(131.00-149.00)
F	3.98±0.80(0.01-5.00)
Troponin	0±1(0-8)
CK-MB	9.54±15.99(1.00-94.85)
APTT	31.38±5.77 (23.00-50.90)
PT	13.83±1.58 (11.00-19.00)
INR	1.07±0.11 (0.89-1.49)

The data obtained at the time of admission of the patients are shown in Table 3. Of the patients, 80% had pain, 10% was vomiting, 50% had edema, 45% had cold extremities, 22.5% was sweating, 2.5% had convulsions, and 7.5% had priapism. When the blood pressure values of the patients were examined, 70% had normal blood pressure, 25% had hypotension, and 5% had hypertension. Of the patients, 70% was conscious at the time of admission, 27.5% was confused, and 2.5% was in a state of coma.Distribution of treatments received by the pediatric patients during the clinical stage and at the time of admission is shown in Table 4. It was observed that 72.5% of the patients

included in the study were Stage 1, and 27.5% was Stage 2 patients. Of the patients, 67.5% was found to have received one dose of antivenomous, and 52.5% of them have received a tetanus vaccine.Biochemistry, coagulation and hemogram results of the patients are shown in Table 5.

Table 6. Evaluation of Homeostasis Measurements of Thiol/Disulfide Homeostasis by Groups

	Patient (n=40)	Control (n=40)	
	Mean±SD	Mean±SD	p
Native Thiol (µmol/l)	345.07±107.08	343.23±90.21	0.934
Total Thiol (µmol/l)	457.08±153.61	398.94±100.38	**0.049**
Disulfide (µmol/l)	56.00±33.60	27.86±11.20	**0.000**
Disulfide/Native Thiol %	17.81±13.48	8.26±3.43	**0.000**
Disulfide/Total Thiol %	12.07±5.87	6.94±2.57	**0.000**
Native Thiol/Total Thiol %	75.87±11.74	86.11±5.13	**0.000**

*p<0.01

In terms of thiol/disulfide homeostasis, mean native thiol levels (-SH) were found to be 345.07±107.08 and 343.23±90.21 mmol/l in the patient and control groups, respectively, and no statistically significant difference was found in terms of mean native thiol levels (p =0.934, p>0.05). There was a statistically significant difference between the two groups in terms of thiol levels (-SH + -S - S-) (p=0.049, p<0.05). Total thiol levels in the patient group (mean 457.08±153.61 mmol/l) were statistically significantly higher than the levels in the control group (mean 398.94±100.38 mmol/l). Similarly, there was a statistically significant difference between the two groups in terms of disulfide (SS) levels (p=0.001, p<0.05). Disulfide levels in the patient group (mean 56.00±33.60) were statistically significantly higher than the levels in the control group (mean 27.86±11.20). The rates of disulfide/native thiol and disulfide/total thiol in the patient group were significantly higher than the control group (p=0.000, p<0.05). A statistically significant difference was found between the two groups in terms of native thiol/total thiol rates (p=0.000, p<0.05). Native thiol/total thiol rate in the patient group was lower than the control group. Thiol/disulfide homeostasis parameters of both groups are shown in Table 6.

DISCUSSION

The scorpion sting incidents are common, especially in the summer and in the Southeastern Anatolia region of Turkey. It is estimated that 1.23 million scorpion sting incidences occur annually in the world, of which about 3250 are fatal (9). Exact figures and mortality rates are unknown as not every case is taken to hospital, and mortality and case records are not kept as required. Mortality rates in children range from 1.9% to 8.9% (10, 11).

Scorpion stings are known to be more severe in children. Mortality and morbidity can be significantly reduced by the timely antivenomous administration, appropriate fluid resuscitation, cardiac monitoring, renal function monitoring (12, 13).

A study in the Diyarbakır region reported that cases of scorpion stings were more common in females (13). In our study, there were more cases of scorpion stings in male children. It is believed that this may be due to the fact that male children in our region go out of the house more easily, starting with the age of play.

Studies report that scorpion sting incidents are more common in children between the ages of 2-5; Altınkaynak et al. (10) have reported in their study that cases of scorpion stings are most common between the ages of 1-10, likewise Osnaya-Romero et al. (14) found that the scorpion sting cases were more common in the 1-9 age group. Adıgüzel et al. (12) reported in their study that the most common admission was in patients between the ages 9 and 15. In our study, the scorpion stings were more common between the ages of 11-15 years (30%). Children are more vulnerable to scorpion stings than adults because of their inexperienced and careless behavior.

Epidemiological studies have reported that the region most bitten is the extremities in cases of scorpion sting (10, 12). In our study, 11 of our patients (27.5%) had a scorpion sting at the right foot. Of the remaining pediatric patients, 22.5% was stung from right hand, 17.5% from left foot, and 12.5% from the hip.

A delay in required medical treatment causes a severe clinical outcome in children (15). Systemic effects depend on the release of catecholamines and acetylcholine. Symptoms will occur in one or two minutes, reaching the highest level in 5 hours and ending in 72 hours. In the autonomic nervous system, especially sympathetic effects (hyperthermia, tachycardia, tachypnea, hypertension, edema,and, hyperglycemia) are at the forefront. Bronchoconstriction, bradycardia, hypotension, and increased secretion throughout the body can also be seen due to parasympathetic influence (16).Although it is not known when the first symptoms started in the patients followed up, manifestation was found to advance in the 0-3 hour period (97%), and 80% of our patients had pain, 10% was vomiting, 50% had edema, 45% had cold extremity, and 22.5% was sweating. Normal blood pressure was present in 70% of patients, 25% had hypotension, and 5% had hypertension.

Dudin et al. (17) have observed improvement in symptoms after performing IV antivenomous treatment upon clinical symptoms such as respiratory distress, brain edema, convulsions in patients they followed after scorpion stings. They also reported that two patients who were not given antivenomous treatment died. They reported more severe condition when the patient's age was smaller, scorpion bites were on the head and neck, or in multiple locations, in addition to poor prognosis in convulsions. In our study, it was found that 2.5% of the patients developed convulsions and 40% of them were evaluated by pediatric cardiology because of arrhythmia.

Priapism is common in ages 5-10, with idiopathic causes being the most common cause (18). It is stated that priapism should be considered as a precursor in terms of systemic symptoms in patients admitted with scorpion sting. Bawaskar et al. (19) reported systemic symptoms and particularly cardiac and lung symptoms following priapism in male patients. In our study, only 3 patients (7.5%) had priapism and cold in the hands and feet, tachycardia and edema were identified in the same patients, and monitored in intensive care.

Clinical signs and symptoms observed in humans and experimental animals were associated with the host's extreme systemic inflammatory response to scorpion sting. Under normal conditions, host cells are protected against the toxic effects of reactive oxygen species by enzymatic and non-enzymatic antioxidants (20). The significant increase in the production of reactive oxygen species during pathological conditions such as acute and chronic inflammation causes oxidative cell and tissue injury when the body's defense mechanisms are surpassed. The term oxidative stress has been adopted to describe the condition resulting in the accumulation of free radicals disabled by molecules known as antioxidants (21).

The thiols, which are the main part of the intracellular and extracellular oxidative stress protection mechanism, bind the free oxidant atoms, make them harmless and are converted to SS as a result of this reaction. Previous studies have shown that oxidative stress increases in patients with scorpion sting (22, 23). Thiol and disulfide parameters, which can be easily measured in serum by a new method developed recently by Erel and Neselioglu(6), are used to obtain information about oxidative stress. In our study, we used this method developed in 2014, which measures thiol and disulfide levels.

There are very few studies on the role of thiol/disulfide homeostasis in the field of pediatrics in the literature. Durmuş et al. (24) found significantly higher rates of disulfide/native thiol and disulfide/total thiol in children with Type 1 diabetes mellitus, while the native thiol and native thiol/total thiol levels were lower in the patient group. Disulfide/native thiol and disulfide/total thiol were reported to be higher, with a balance shifted in favor of disulfide. Similarly, in the study by Ozdamar et al. (25) conducted with pediatric adenoid hypertrophy patients, the values of disulfide, disulfide/native thiol and disulfide/total thiol were found to be higher. In these patients, the natural thiol and total thiol values were reported to be lower than the healthy group.

In our study, we evaluated the thiol/disulfide homeostasis in children exposed to scorpion sting. There was no statistically significant difference between the two groups in terms of native thiol levels Total thiol, disulfide, disulfide/native thiol rate and disulfide/total thiol rate were found to be significantly higher in the patient group, while the rate of native thiol/total thiol was statistically significantly lower in the patient group than in the healthy group. In our study, we found that thiol/disulfide homeostasis shifts to the disulfide side, i.e. to the right, and oxidative stress increases in children exposed to scorpion sting. The increased disulfide, disulfide/total thiol rate (oxidized thiol ratio), and disulfide/native thiol rate (thiol oxidation/reduction ratio) indicate an increase in oxidative stress, whereas low native thiol/total thiol rate compared to healthy children indicates a low antioxidant defense mechanism.

CONCLUSION

In children with scorpion sting, the thiol/disulfide homeostasis was observed to shift towards the disulfide side, i.e. to the right, indicating an increase in oxidative stress. Increased disulfide, oxidized thiol ratio and thiol oxidation/reduction ratio indicate increased oxidative stress, whereas a native thiol rate similar to that of healthy children and low Native Thiol/Total Thiol ratio in the patient group indicate a low antioxidant defense mechanism.

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